Optical Absorption and EPR Studies on a Six-coordinate Iron(III)–tetramesitylporphyrin–Hydrogen Peroxide Complex Having a Nitrogenous Axial Ligand

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The formation of a six-coordinate Fe^{III}TMP–hydrogen peroxide complex (TMP = tetramesitylporphyrin), having a nitrogenous ligand at the axial position, is demonstrated by means of simultaneous EPR and optical absorption measurements at 77 K.

The coordination and electronic structures of haem–hydrogen peroxide complexes have attracted the interest of many chemists and biochemists, in relation to the biological functions of haem enzymes, such as cytochrome c peroxidase[1] and horseradish peroxidase.[2] So far, the mechanisms of the reaction between model haem complexes and hydrogen peroxide[3,4] have been studied thoroughly by several groups, but little is yet known about the coordination chemistry of haem–hydrogen peroxide complexes, since they have very short life-time and are too unstable to be detected by usual spectroscopic methods. By means of simultaneous EPR and optical absorption measurements at 77 K, with the aid of freeze–thaw methods, we would like to demonstrate the formation of a six-coordinate Fe^{III}TMP–hydrogen peroxide complex, having a nitrogenous axial ligand derived from imidazole.

According to the usual methods,[5] Fe^{III}TMPCl, Fe^{III}TPPCl (TPP = tetraphenylporphyrin) and Fe^{III}OEPCl (OEP = octaethylporphyrin), were prepared in our laboratory and purified by silica gel column chromatography. All the sample solutions for the simultaneous EPR and optical measurements were prepared in a standard EPR quartz tube (5.0 mm diameter) or in an EPR flat cell at −60 °C, in order to prevent oxidative decomposition of the iron–porphyrin complexes. To a pre-cooled reaction mixture (−60 °C) composed of an aqueous KOH solution (1.0 mol dm^{-3}, 0.01 ml) and a DMF–methanol–toluene mixed solution (90:5:5 *v/v*) of Fe^{III}TMPCl (1.0 mmol dm^{-3}, 0.4 ml), a DMF–water mixed solution (50:50 *v/v*) of hydrogen peroxide (1.0 mol dm^{-3}, 0.04 ml) was added rapidly and immediately frozen at 77 K. The optical absorption spectrum (Ohtsuka Electronics MCPD-1000) exhibited the Soret absorption maxima at 428 nm with a well-formed Q-band doublet with maxima at 563 and 601 nm, as shown in Fig. 1(*a*). The EPR spectrum of the same frozen solution [Fig. 2(*a*); JEOL FE2XG X-band spectrometer]‡ showed the presence of a ferric low-spin species (denoted as complex A; g_1 = 2.257, g_2 = 2.156 and g_3 = 1.963). The formation of similar ferric low-spin species were also detected for both Fe^{III}TPPCl and Fe^{III}OEPCl by the same reaction conditions (Table 1). In comparison of the observed EPR and optical parameters and those of relating hydrogen peroxide complexes[3] (Table 1), the axial ligands of these complexes A are safely assigned to be the $^-$OH and $^-$OOH derived from aqueous KOH and hydrogen peroxide, respectively (Scheme 1).

Then, the frozen solution of the complex A of Fe^{III}TMP was thawed at −60 °C, and a DMF solution of imidazole (0.5 mol dm^{-3}, 0.04 ml; molar ratio Fe^{III}TMP : imidazole adjusted to 1 : 25) was rapidly mixed in and the mixture refrozen within 2 min. As shown in Fig. 2(*b*), the observed spectrum revealed the formation of a new low-spin ferric species (denoted as complex B; g_1 = 2.32, g_2 = 2.20 and g_3 = 1.93), with the remaining signals due to complex A. When the reaction mixture was treated with a 15 molar excess amount of imidazole, the apparent EPR intensity of complex B (g_1 = 2.320, g_2 = 2.191 and g_3 = 1.943) reached a maximum, while that of complex A disappeared completely. The same frozen solution showed Soret and Q-band absorption maxima at 424, 557 and 599 nm, respectively, as shown in Fig. 1(*b*). It should be noted here that

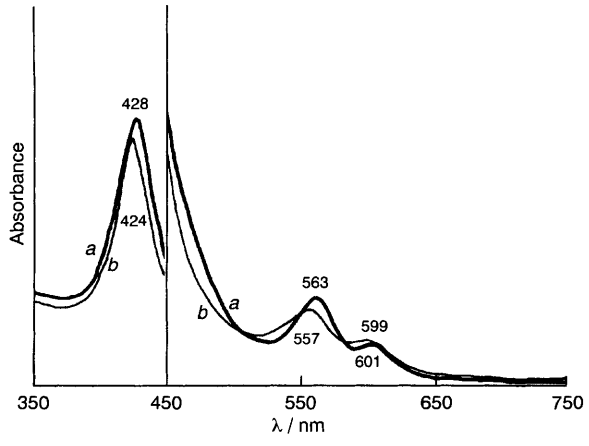


Fig. 1 Optical absorption spectra recorded at 77 K during the titration of the Fe^{III}TMP($^-$OH)($^-$OOH) complex against imidazole. (*a*) Spectrum of Fe^{III}TMP($^-$OH)($^-$OOH) prepared by mixing Fe^{III}TMPCl, hydrogen peroxide and KOH; (*b*) spectrum recorded after the addition of a 50 molar excess amount of imidazole to the solution of (*a*).

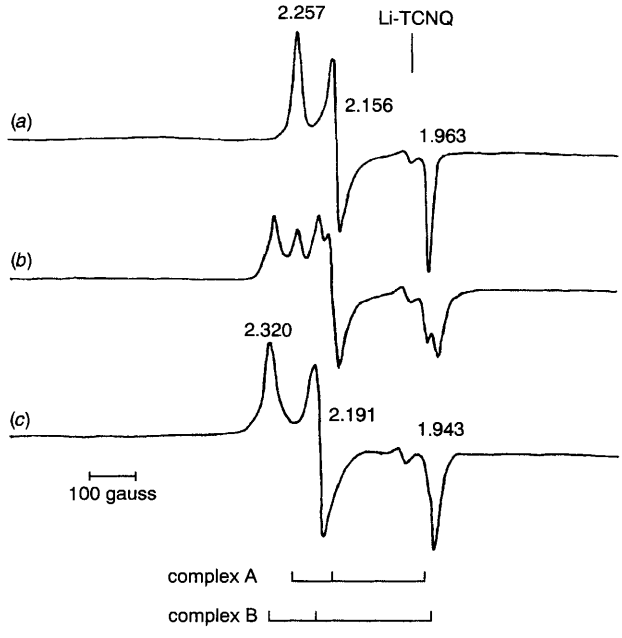
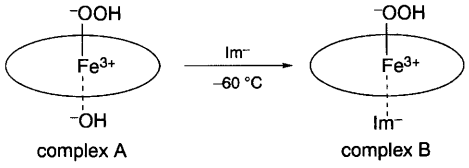


Fig. 2 EPR spectra recorded for the same frozen solutions supplied for optical absorption measurements at 77 K during the titration of Fe^{III}TMP($^-$OH)($^-$OOH) complex by imidazole. (*a*) Spectrum recorded for Fe^{III}TMP($^-$OH)($^-$OOH) complex; (*b*) spectrum recorded after the addition of a 25 molar excess amount of imidazole to the solution of (*a*); (*c*) spectrum recorded after the addition of a 15 molar excess amount of imidazole to the solution of (*b*).

Table 1 EPR and optical parameters of haem–hydrogen peroxide and related low-spin complexes

Complexes	Peroxide	Base	λ_{max}/nm	*g* values			Crystal field parameters		Ref.
				g_1	g_2	g_3	$\lvert\lambda/\mu\rvert$	$\lvert R/\mu\rvert$	
$Fe^{III}TMP(^-OH)(^-OOH)$	HOOH	KOH	428,563,601	2.257	2.156	1.963	10.0	0.44	*g*
$Fe^{III}TPP(^-OH)(^-OOH)$	HOOH	KOH	420,562,605	2.264	2.157	1.962	9.8	0.45	*g*
$Fe^{III}TPP(^-OH)(^-OOH)$	HOOH	TPA[a]	420,562,605	2.274	2.163	1.959	9.0	0.45	3
$Fe^{III}OEP(^-OH)(^-OOH)$	HOOH	KOH	556,604	2.287	2.171	1.955	8.9	0.44	*g*
$Fe^{III}OEP(^-OH)(^-OOH)$[b]	O_2	AscN[c]	556,604	2.286	2.171	1.953	8.9	0.44	3
$Fe^{III}TMP(Im)(^-OOH)$	HOOH	KOH, Im	424,557,599	2.320	2.191	1.943	8.0	0.44	*g*
$Fe^{III}Hb(^-OOR)$[d,e]	Bu^tOOH	TMAOH[f]	421,548,581	2.340	2.188	1.940	7.9	0.51	10
$Fe^{III}Hb(^-OOR)$[d,e]	Bu^nOOH	TMAOH[f]	421,548,581	2.343	2.190	1.933	7.7	0.50	10
$Fe^{III}Hb(^-OH)$[e]	none		—	2.65	2.19	1.83	5.8	0.53	8

[a] TPA, tri-n-propylamine. [b] Prepared by reduction of the oxygen complex with the sodium salt of ascorbic acid. [c] AscNa, sodium salt of ascorbic acid. [d] ^-OOR is the deprotonated form of *tert*-butylhydroperoxide. [e] Hb, haemoglobin [f] TMAOH, tetramethyammonium hydroxide dimethyl ester. [g] This work.



Scheme 1 Possible axial ligand exchange reaction occurring between $Fe^{III}TMP(^-OH)(^-OOH)$ and imidazole. The oval corresponding to the dianion form of TMP.

a weak EPR signal detected in a lower magnetic field (g = 4.3) can be ascribable to small amounts of non-haem ferric species which form from $Fe^{III}TMP$ *via* oxidative decomposition by reaction with hydrogen peroxide. In contrast to the case of $Fe^{III}TMP$, EPR signals due to the complexes A of both $Fe^{III}TPP$ and $Fe^{III}OEP$ were completely changed to the non-haem iron species at g = 4.3, and the Soret band absorption maximum at 424 nm also disappeared, after the addition of a 25 molar excess amount imidazole at −60 °C. These observations suggest that complexes A derived from $Fe^{III}TPP$ and $Fe^{III}OEP$ can decompose to open-chain polypyrrole iron(III) species, during the freeze–thaw treatment at −60 °C. To our knowledge, EPR and optical absorption spectra due to complex B are observed only for $Fe^{III}TMP$, which probably possesses sufficient stability against the oxidative decomposition promoted by hydrogen peroxide.

The EPR and optical spectra changes, observed before and after the addition of imidazole to complex A of $Fe^{III}TMP$, are indicative that one of the axial ligands can be replaced with a nitrogenous ligand derived from imidazole. The probable coordination structure of complex B is assumed to be either $Fe^{III}TMP$–(imidazole)(^-OOH) or $Fe^{III}TMP$–(imidazole)-(^-OH). In comparing the g-parameters of complex B and six-coordinate iron(III)–hydroxide complexes, such as hydroxy-met-myoglobin and -haemoglobin complexes[6] (Table 1), we found the g_1–g_3 separation of complex B to be distinctively smaller than those iron(III) hydroxide complexes. Furthermore, it has been frequently demonstrated that the axially binding hydroxide anion of iron(III)–bleomycin[7] and –azacrown macrocycles[8] is easily replaced by the hydrogen peroxide anion under alkaline conditions. The six-coordinate $Fe^{III}TMP$(imidazole)(^-OH) type structure is ruled out as a candidate for the structure of complex B. In order to obtain information about whether the peroxide anion ligated at the axial position, the crystal field parameters of complex B and related complexes were calculated after Bohan's proposal.[9] As summarized in Table 1, the larger than usual tetragonality of complex B (8.0) compared with other iron complexes is characteristic of six-coordinate haem–peroxide complexes, having a peroxide anion derived from either hydrogen peroxide or organic peroxide. In addition, the tetragonality ($\lvert\lambda/\mu\rvert$) and rhombosity ($\lvert R/\mu\rvert$) parameters of complex B are consistent with those of met-haemoglobin and -myoglobin peroxide complexes,[10] in which the nitrogenous donor derived from histidine is retained at the axial position of haem (Table 1). Accordingly, the hydroperoxide anion and nitrogenous ligand of imidazole are proposed to be the possible axial ligands of complex B. Therefore, the coordination structure of the complex is tentatively formulated as the six-coordinate $Fe^{III}TMP$(imidazole)(^-OOH). The observed optical absorption and EPR spectral changes (Figs. 1 and 2) can be interpreted as an axial ligand-exchange reaction between imidazole and the hydroxide anion, occurring at the fifth position of complex A. These findings described herein proved that sterically hindered $Fe^{III}TMP$ is a suitable iron complex for studying the electronic and coordination structures of the haem–hydrogen peroxide complex.

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Footnotes

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‡ The g-values of the observed EPR spectra were estimated by using the lithium salt of tetracyanoquinodimethane (Li-TCNQ; g = 2.0025) as an external standard. The microwave frequency for EPR excitation was monitored using an Advantec TR-5211 digital frequency counter.

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